REMARKS

Claims 1-3, 6-21 and 24-72 are pending in this application. Pending claims 1-3, 6, 8-14, 16-21, 24-26, 28-31, 33-37, 39-49, 51-67 and 70-71 have been indicated to be allowable. Pending claims 7, 32, 38, 50, 68, 69 and 72 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 66 is objected to on grounds of inconsistency. The rejections and objections are addressed by the above amendments and the following arguments.

The Amendments

Claims 7, 50, 66 and 68-69 are amended to correct inadvertant and obvious typographical errors; grammatical errors were introduced by amendment of claims 7, 50, 66 and 68-69 on January 26, 2001, and these errors are corrected herein. Claim 15 is amended at the request of the Examiner to insert the definition of the standard abbreviation for the well known intermediate in ethylene biosynthesis, as exemplified in passages from standard textbooks submitted with the amendment of October 4, 2001. Claim 32 is amended to insert definitions for well known compounds which were listed in the originally filed application by their trade names, as shown in the attached passages from "The Merck Index, 12th edition, pages 365 and 986, a Material Safety Data Sheet for chlorflurenol and catalog entries from the Sigma Chemical Catalog.

The above amendments do not change the scope of claims. Applicants submit that these amendments are fully supported in the application as filed and add no new matter to the application. The amendments are made at this time to address issues first raised in the Office Action dated December 19, 2001, and were not made earlier, because the first indication to Applicants that amendment would be needed was in that Office Action. Therefore entry of the amendments under 37 C.F.R. § 1.116(b) is proper. Entry of these amendments is respectfully requested.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 7, 32, 38, 50, 68, 69 and 72 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed.

The issues raised by rejection of claims 7, 15, 32, 50, and 68-69 are addressed by the present amendments, which do not narrow the scope of the claims. Applicants are unable to determine the basis for rejection of claim 38, and so that claim remains unchanged. If the Examiner intends to maintain rejection of claim 38, Applicants respectfully request that the grounds for such rejection be expressly set forth. Applicants also request that any grounds for rejection of Claim 27 also be set forth.

Claim 72 as amended on October 4, 2001, recites that β–phenylalanine is added to one or more of the nutrient media in an amount sufficient to enhance taxane production. The specification describes and exemplifies detection of taxanes produced in cell cultures according to this invention, and it is a routine matter for the ordinary worker to titrate any component of the nutrient medium to determine the level of the component at which the amount of taxane produced in a culture is enhanced over a control culture which does not contain the component. For the enhancing precursor material described on page 25, lines 26-27, the amount used to provide useful enhancement to taxane production will depend on the culture conditions as taught in the specification on page 21, lines 9-28, and the particular amount of ß-phenylalanine for particular culture conditions may be determined by the skilled worker in view of the guidance in the specification. Claim 72 distinctly claims a method for producing taxanes in which ß-phenylalanine is present in the culture in at least the amount determined by the ordinary worker in this manner. Therefore, this claim complies with the requirements of 35 U.S.C. § 112, second paragraph.

A clean copy of Table 2 was submitted on October 4, 2001, as indicated by the U.S. Patent Office Mail Room stamp on the receipt postcard (copy enclosed herewith). Another copy of Table 2 is enclosed with this submission.

It is believed that the above amendments and arguments, and the enclosed copy of Table 2, address all issues raised in rejection of claims 7, 32, 38, 50, 68, 69 and 72 under 35 U.S.C. § 112, second paragraph. Therefore, Applicants respectfully request that the rejection of all claims be withdrawn.

Applicants believe that the subject application is now in condition for allowance, and such disposition is earnestly solicited. If the Examiner believes that prosecution might be furthered by discussing the application with Applicants' representative, in person or by telephone, we would welcome the opportunity to do so.

Respectfully submitted,

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APPENDIX A
VERSION OF CLAIMS WITH MARKINGS
U.S. Application No. 08/479,809
(as amended March 2002)

In accordance with 37 C.F.R. § 1.121(c), Applicants submit a marked-up version of the claims, in order to indicate changes Applicants have made.

AMENDED CLAIMS

1. (thrice amended) A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein at least one of the one or more nutrient media comprises one or more enhancement agents selected from the group consisting of (a) jasmonate-related compounds or alkyl esters thereof, (b) antiethylene agents, and (c) inhibitors of phenylpropanoid metabolism.

2. The method of claim 1, wherein the one or more nutrient media contain an antiethylene agent which is a silver-containing compound, or a silver complex, or a silver ion.

3. (twice amended) The method of claim 1, wherein a jasmonate-related compound or an alkyl ester thereof is added to the one or more nutrient media.

6. (amended) The method of claim 3, wherein the jasmonate-related compound is in a concentration from 10^{-5} to $2x10^{-4}$M.

7. (twice amended) The method of claim 3, wherein the jasmonate-related compound is at least one compound selected from the group consisting of jasmonic acid[,] and dihydrojasmonic acid.

8. The method of claim 3, wherein the jasmonate-related compound is at least one compound selected from the group consisting of jasmonic acid and alkyl esters of jasmonic acid.

9. (twice amended) The method of claim 8, wherein said alkyl ester of jasmonic acid comprises an alkyl group esterified to jasmonic acid wherein said alkyl group has from one to four carbon atoms.

10. The method of claim 8, wherein the alkyl group esterified to jasmonic acid has one carbon atom.

11. The method of claim 3, wherein the cells are cultured in the presence of heavy metal ions, heavy metal complexes, or heavy metal-containing compounds.

12. (amended) The method of claim 11, wherein the heavy metal ions are cobalt ions, the heavy metal complexes are cobalt complexes, and the heavy metal-containing compounds are cobalt-containing compounds.

13. (amended) The method of claim 3, wherein the cells are cultured in the presence of an antiethylene agent.

14. The method of claim 13, wherein the antiethylene agent is an ethylene-biosynthesis antagonist.

15. (amended) The method of claim 14, wherein the ethylene-biosynthesis antagonist is a compound which inhibits aminocyclopropane carboxylic acid (ACC) synthase, ACC oxidase, or ethylene oxidase.

16. The method of claim 14, wherein the ethylene-biosynthesis antagonist is acetylsalicylic acid or aminooxyacetic acid.

17. The method of claim 13, wherein the antiethylene agent is an ethylene-action antagonist.

18. The method of claim 17, wherein the ethylene-action antagonist is a silver-containing compound, a silver complex or silver ion.

19. (amended) The method of claim 18, wherein the silver-containing compound is at least one compound selected from the group consisting of silver thiosulfate, silver chloride, and silver oxide.

20. (amended) The method of claim 18, wherein the silver-containing compound is at least one compound selected from the group consisting of silver phosphate, silver benzoate, toluenesulfonic acid silver salt, silver acetate, silver nitrate, and silver sulfate.

21. (amended) The method of claim 18, wherein the silver-containing compound is at least one compound selected from the group consisting of silver pentafluoropropionate, silver cyanate, lactic acid silver salt, silver hexafluorophosphate, citric acid trisilver salt, and silver nitrite.

24. (twice amended) The method of claim 18, wherein the concentration of silver ions, silver complexes, or silver-containing compounds is 10 μM – 100 μM

25. (twice amended) The method of claim 18, wherein the concentration of silver ions, silver complexes, or silver-containing compounds is 50 μM.

26. (twice amended) The method of claim 18, wherein the concentration of silver ions, silver complexes, or silver-containing compounds is 10 μM.

27. (amended) The method of claim 18, wherein silver and jasmonate are present in the one or more nutrient media in molar ratio of silver: jasmonate of less than 9.5.

28. The method of claim 1, wherein the one or more nutrient media contain an inhibitor of phenylpropanoid metabolism.

29. (amended) The method of claim 28, wherein the inhibitor of phenylpropanoid metabolism is selected from the group consisting of 3,4,-methylenedioxynitrocinnamic acid 3,4-methylenedioxycinnamic acid, 3,4-methylenedioxy-phenylpropionic acid, 3,4-methylenedioxyphenylacetic acid, 3,4-methylenedioxybenzoic acid, 3,4,-trans-dimethoxycinnamic acid, 4-hydroxycinnamic acid, phenylpropionic acid, fluorophenylalanine, 1-aminobenzotriazole, 2-hydroxy-4,6-dimethoxybenzoic acid, 2-(diethylamino)ethyl ester of α-phenyl-α-propylbenzeneacetic acid, ammonium oxalate, vinylimidazole, diethyldithiocarbamic acid, and sinapic acid.

30. The method of claim 1, wherein the one or more nutrient media contain at least one enhancement agent selected from each of at least two of the following classes of enhancement agents: (a) jasmonic acid or an alkyl ester thereof, (b) antiethylene agents, and (c) inhibitors of phenylpropanoid metabolism.

31. The method of claim 30, wherein the jasmonic acid alkyl ester is methyl jasmonate.

32. (amended) The method of claim 1 or claim 30, wherein the one or more nutrient media further comprise an auxin-related growth regulator selected from the group

consisting of 1-Naphthaleneacetic acid, 2-Naphthaleneacetic acid, 1-Naphthaleneacetamide/ Naphthylacetamide, N-(1-Naphthyl)phthalamic acid, [,]1-Naphthoxyacetic acid, 2-Naphthoxyacetic acid, beta-Naphthoxyacetic acid, 1-Naphthoxyacetamide,, 3-Chlorophenoxyacetic acid, 4-Chlorophenoxyacetic acid, 4-Iodophenoxyacetic acid, Indoleacetamide, Indoleacetic acid , Indoylacetate, Indoleacetyl leucine, Gamma-(3-Indole)butyric acid, 4-Amino-3,5,6-trichloropicolinic acid, 4-Amino-3,5,6-trichloropicolinic acid methyl ester, 3,6-Dichloro-o-anisic acid, 3,7-Dichloro-8-quinolinecarboxylic acid, Phenylacetic acid, 2-Iodophenylacetic acid, 3-Iodophenylacetic acid, 2-Methoxyphenylacetic acid, Chlorpropham (m-chlorocarbanilic acid isopropyl eser), 4-chloroindole-3-acetic acid, 5-Chloroindole-3-acetic acid, 5-Bromo-4-chloro-3-indoyl butyrate, Indoleacetyl phenylalanine, Indoleacetyl glycine, Indoleacetyl alanine, 4-chloroindole, p-chlorophenoxyisobutyric acid, 1-pyrenoxylbenzoic acid, Lysophosphatidic acid, 1-naphthyl-N-methylcarbamate, Ethyl-5-chloro-1H-Indazole-3-ylacetate-3-Indolebutanoic acid, Naphthalene-2,6-dicarboxylic acid, Naphthalene-1,4,5,8-tetracarboxylic acid dianhydride, Naphathalene-2-sulfonamide, 4-Amino-3,6-disulfo-1,8-naphthalic anhydride, 3,5-dimethylphenoxyacetic acid, 1,8-Naphthalimide, 2,4-Dichlorophenoxyacetic acid, 2,3-Dichlorophenoxyacetic acid, 2,3,5-Trichlorophenoxyacetic acid, 2-Methyl-4-chlorophenoxyacetic acid, Nitrophenoxyacetic acids, DL-alpha-(2,4-Dichlorophenoxy)propionic acid, D-alpha-(2,4-Dichlorophenoxy)propionic acid, 4-Bromophenoxyacetic acid, 4-Fluorophenoxyacetic acid, 2-Hydroxyphenoxyacetic acid, 5-Chloroindole, 6-Chloro-3-indoylacetate, 5-Fluoroindole, 5-Chloroindole-2-carboxylic acid, 3-Chloroindole-2-carboxylic acid, Indole-3-pyruvic acid, 5-Bromo-4-chloro-3-indoylbutyrate, 6-Chloro-3-indoylbutyrate, Quinoline-2-thioglycolic acid, Aminophenylacetic acids, 3-Nitrophenylacetic acid, 3-Chloro-4-hydroxybenzoic acid, Chlorflurenol (2-chloro-9-hydroxyfluorene-9-carboxylic acid), 6-Chloro-3-indoyl acetate, N-(6-aminohexyl)-5-chloro-1-Naphthalenesulfonamide hydrochloride, 2-chloro-3(2,3-dichloro-phenyl) propionitrile, o-chlorophenoxyacetic acid, 6,7-dimethoxy-1,2-benzisoxazole-3-acetic acid, 3-oxo-1,2,-benzisothiazoline-2-ylacetic acid, Mastoparan (insect venom tetradeca peptide), 2,3,5-Triidobenzoic acid, 2-(3-chlorophenoxy)propanoic acid, Mecoprop (2-(4-chloro-2-methylphenoxy)-propanoic acid), Naphthoic acid hydrazide, 2,4-Dibromophenoxyacetic acid, 3-Trifluoromethylphenoxyacetic acid, Oxindole, Indole-2-carboxylic acid, Indole-3-lactic acid,

Beta-(3-Indole)propionic acid, 2-Bromophenylacetic acid, 3-Bromophenylacetic acid, 2-Chlorophenylacetic acid, 3-Chlorophenylacetic acid, 2-Methylphenylacetic acid, 3-Methylphenylacetic acid, 3-Trifluoromethylphenylacetic acid, 3-Methylthiophenylacetic acid, Phenylpropionic acid, 4-chloro-2-methylphenylthioacetic acid, 2-Chlorobenzoic acid, 3-Chlorobenzoic acid, 2,3-Dichlorobenzoic acid, 3,4-Dichlorobenzoic acid, 2,3,5-Trichlorobenzoic acid, 2,4,6-Trichlorobenzoic acid, 2-Benzothiazoleoxyacetic acid, 2- Chloro-3-(2,3-dichlorophenyl)propionitrile, 2,4-Diamino-s-triazine, Naphthalic anhydride, Dikegulac, chlorflurecolmethyl ester, 2-(p-chlorophenoxy)-2-methylpropionic acid, 2-chloro-9-hydroxyfluorene-9-carboxylic acid, 2,4,6-trichlorophenoxyacetic acid, 2-(p-chlorophenoxy)-2-methyl propionic acid, Ethyl 4-(chloro-o-tolyloxy)butyrate, [N-(1,3-dimethyl-1H-Pyrazol-5-yl)-2-(3,5,6-Trichloro-2-pyridinyl)oxy]acetamide, 4-Chloro-2-oxobenzothiazolin-3-yl-acetic acid, 2-(2,4-Dichlorophenoxy)propanoic acid, 2-(2,4,5-Trichlorophenoxy) propanoic acid, 4-Fluorophenylacetic acid, 3-Hydroxyphenylacetic acid, Orthonil, 3,4,5-Trimethoxycinnamic acid, 2(3,4-dichlorophenoxy)triethylamine, Indole-3-propionic acid, Sodium Ioxynil, 2-Benzothiazoleacetic acid, and (3-phenyl-1,2,4-thiadiazol-5-yl)thioacetic acid.

33. The method of claim 30, wherein the antiethylene agent is a silver-containing compound, a silver complex or silver ion.

34. (amended) The method of claim 30, wherein the inhibitor of phenylpropanoid metabolism is selected from the group consisting of 3,4,-methylenedioxynitrocinnamic acid, 3,4,-methylenedioxycinnamic acid, 3,4,-methylenedioxy-phenylpropionic acid, 3,4,-methylenedioxyphenylacetic acid, 3,4-methylenedioxybenzoic acid, 3,4,-trans-dimethoxycinnamic acid, 4-hydroxycinnamic acid, phenylpropionic acid, fluorophenylalanine, 1-aminobenzotriazole, 2-hydroxy-4,6-dimethoxybenzoic acid, 2-(diethylamino)ethyl ester of α-phenyl-α-propylbenzeneacetic acid, ammonium oxalate, vinylimidazole, diethyldithiocarbamic acid, and sinapic acid.

35. (amended) The method of claim 1, claim 3, or claim 30, wherein the one or more nutrient media further comprises a polyamine.

36. The method of claim 35, wherein the polyamine is selected from the group consisting of spermine, spermidine, putrescine, cadaverine, and diaminopropane.

37. The method of claim 1 or claim 30, wherein the one or more nutrient media further comprise a taxane precursor.

38. The method of claim 32, wherein the auxin-related growth regulator is picloram, indoleacetic acid, 1-naphthaleneacetic acid, indolebutyric acid, 2,4-dichlorophenoxyacetic acid, 3,7-dichloro-8-quinolinecarboxylic acid, or 3,6-dichloro-o-anisic acid.

39. (amended) The method of claim 1, wherein the amount of said one or more taxanes recovered is at least 3-fold greater than the amount obtained from cells of Taxus species cultured without addition of any enhancement agents selected from the group consisting of (a) jasmonate-related compounds or alkyl esters thereof, (b) anti-ethylene agents, and (c) inhibitors of phenylpropanoid metabolism.

40. (amended) The method of claim 1, wherein the amount of said one or more taxanes recovered is at least 5-fold greater than the amount obtained from cells of Taxus species cultured without addition of any enhancement agents selected from the group consisting of (a); jasmonate-related compounds or alkyl esters thereof, (b) anti-ethylene agents, and (c) inhibitors of phenylpropanoid metabolism.

41. (twice amended) The method of claim 1, wherein said one or more taxanes recovered is at least one compound selected from the group consisting of taxol, 7-epitaxol, 10-deacetyl-7-epitaxol, cephalomannine, 10-deacetyltaxol, 7-xylosyl-10-deacetyltaxol, baccatin III, and 10-deacetylbaccatin III.

42. (twice amended) The method of claim 1, wherein the cells are cultured in a first medium having a first composition, then the medium composition is changed to a second medium having a second composition which induces taxane production.

43. (twice amended) The method of claim 42, wherein the concentration of nitrate is lower in the second medium than in the first medium, and the concentration of a saccharide is higher in the second medium than in the first medium.

44. (amended) The method of claim 43, wherein the first medium contains nitrate at a concentration which is 2 to 10 times the nitrate concentration in the second medium.

45. (twice amended) The method of claim 42, wherein the second medium contains a saccharide at a concentration which is 2 to 5 times the saccharide concentration in the first medium.

46. (amended four times) The method of claim 1, wherein the cells are cultured in media containing a saccharide in a concentration of 1 – 150 g/L, nitrate ion in a concentration of 0.3 – 70 mM or a combination thereof.

47. (thrice amended) The method of claim 43, wherein the first medium contains a saccharide [n] in the concentration of 1 – 30 g/L, and nitrate ion in the concentration of 2.5 – 70 mM; and the second medium contains a saccharide in the concentration of 4 – 150 g/L, and nitrate ion in the concentration of 0.3 – 18 mM.

48. (twice amended) The method of claim 43, wherein the first medium contains a saccharide in the concentration of 5 – 15 g/L, and nitrate ion in the concentration of 20 – 30 mM; and the second medium contains a saccharide in the concentration of 35 – 55 g/L, and nitrate ion in the concentration of 2 – 7 mM.

49. (twice amended) The method of claim 42, wherein the medium which induces taxane production is replenished during cultivation by periodically replenishing nutrient medium components and removing spent medium.

50. (twice amended) The method of claim 1 or claim 30, wherein[said] the medium which induces taxane production is replenished during cultivation by periodically replenishing nutrient medium components and removing spent medium.

51. The method of claim 1 or claim 30, wherein nutrient medium is the same for cell culture growth and for taxane production.

52. The method of claim 1 or claim 30, wherein cells of said *Taxus* species are cultivated by a continuous or semi-continuous process.

53. (amended) The method of claim 1, claim 3, or claim 30, wherein cells of said *Taxus* species are cultivated by a fed-batch process.

54. (twice amended) The method of claim 53, wherein the culture medium is replenished during cultivation by periodically replenishing nutrient medium components and removing spent medium.

55. (amended) The method of claim 1 or claim 30, further comprising the periodic removal of said at least one or more taxanes from the nutrient media.

56. The method of claim 1 or claim 30, wherein the *Taxus* species is selected from the group consisting of *T. canadensis, T. chinensis, T. cuspidata, T. baccata, T. globosa, T. floridana, T. wallichiana,* and *T. media.*

57. The method of claim 3 or claim 30, wherein the *Taxus* species is *Taxus brevifolia.*

58. The method of claim 1, wherein the cells are cultured in the presence of 0.03% to 15% v/v of carbon dioxide in the gas phase in equilibrium with the culture medium.

59. (amended) The method of claim 1 or claim 3, wherein the cells are cultured in the presence of 0.3% to 8% v/v of carbon dioxide in the gas phase in equilibrium with the culture medium.

60. The method of claim 1, wherein the cells are cultured in the presence of controlled oxygen concentration between 1% to 200% of air saturation.

61. The method of claim 1, wherein the cells are cultured in the presence of controlled oxygen concentration between 10% to 100% of air saturation.

62. (amended) The method of claim 1 or claim 3, wherein the cells are cultured in the presence of controlled oxygen concentration between 25% to 95% of air saturation.

63. The method of claim 42, wherein the second medium comprises a jasmonate-related compound or an alkyl ester thereof.

64. The method of claim 1 or claim 30, wherein a jasmonate-related compound or an alkyl ester thereof is added continuously to the cell culture.

65. The method of claim 1 or claim 30, wherein the one or more nutrient media contain glutamine.

66. (twice amended) The method of claim 3, wherein the cells are cultured in media containing a saccharide in a concentration of 1 – 150 g/L, nitrate ion in a concentration of 0.3 – 70 mM or a combination thereof.

67. The method of claim 1, wherein the one or more nutrient media contain an antiethylene agent.

68. (twice amended) A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said

medium of said cell culture, or both, wherein at least one of the one or more nutrient media comprises a [compound selected from the group consisting of polyamines]polyamine.

69. ([twice]thrice amended) The method of claim 68, wherein said [polyamines]polyamine [are]is added to at least one of the one or more nutrient media.

70. A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein cells of said *Taxus* species are cultured in the presence of controlled oxygen concentration between 10% to 100% of air saturation.

71. (amended) The method of claim 2, wherein the concentration of silver ions, silver complexes, or silver-containing compounds is 0.01 μM – 10 μM.

72. (amended) A method for producing one or more taxanes in high yields in cell culture of a Taxus species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a Taxus species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein β-phenylalanine is added to the one or more nutrient media in an amount sufficient to enhance taxane production.